REMARKS

Claims 25-42 have been canceled and claims 43-62 have been added. The added claims are fully supported by the specification and claims as originally filed. In the March 18, 2010, office action claims 25-27, 32-36, 38, and 42 were rejected under 35 USC §103(a) as obvious over Li in view of Lopez Garcia and Stemmer. Claims 25-28, 32-38, and 42 were rejected under §103(a) as obvious over Li, Lopez Garcia, Stemmer and Blaschke. Claims 25-27, 29-36, and 38-42 were rejected under §103(a) as obvious over Li, Lopez Garcia, Stemmer, Kyle, Sigma catalog (1998) and Wierenga. The specific grounds for rejection, and applicants' response thereto, are set forth in detail below.

Rejections Under 35 U.S.C. § 103(a)

Claims 25-27, 32-36, 38, and 42 are rejected under 35 USC §103(a) as obvious over Li in view of Lopez Garcia and Stemmer. Claims 25-28, 32-38, and 42 are rejected under §103(a) as obvious over Li, Lopez Garcia, Stemmer and Blaschke. Claims 25-27, 29-36, and 38-42 are rejected under §103(a) as obvious over Li, Lopez Garcia, Stemmer, Kyle, Sigma catalog (1998) and Wierenga. Although the office action sets forth these rejections separately, each rejection rests on an identical set of primary references (Li, Lopez Garcia and Stemmer) with the secondary references (Blaschke, Kyle, Sigma catalog and Wierenga) cited only to show specific limitations set forth in the claims. Accordingly, applicants address below the combination of the primary references and explain how this combination fails to support a *prima facie* case of obviousness.

Li is cited to show that quantitative real time PCR (qRT-PCR) was known, and also that mastermixes for qRT-PCR were known. As such, Li merely confirms what is discussed in the background section of the instant application. The Examiner admits that Li fails to teach or suggest use of antifoams to solve the problems in optical detection of qRT-PCR but asserts that this deficiency is remedied by Lopez Garcia, which is cited as teaching the use of antifoams to improve reproducibility of results in an (unrelated) optical detection method. Stemmer is cited as teaching that anti-foam agents do not substantially inhibit a polymerase during PCR. Applicants respectfully submit that the Examiner's assertions regarding both Lopez Garcia and Stemmer are based upon misreadings of the references and that neither reference supports the arguments proposed by the Examiner.

None of the references addresses the requirement for detergent for polymerase stability

As applicants previously pointed out, it is well known that thermostable DNA polymerases, such as *Taq* polymerase, of the type that are used in qRT-PCR require the presence of non-ionic detergents for stability at higher temperature. See Gelfand, US Patent No. 6,127,155. It is also well known that antifoam compounds control foaming caused by detergents by interfering with detergent action. Accordingly, one skilled in the art would have had no motivation to add antifoam compounds to a qRT-PCR reaction because it would have been expected that the antifoam would have counteracted the beneficial, even necessary action of the detergent and this would have had an adverse effect on polymerase stability, polymerase activity and PCR performance. Higuchi and Gelfand first described qRT-PCR methods with optical detection in 1991[1] and these methods rapidly became among the most widely used techniques in molecular biology. The instant application has a priority date of 2002, over 11 years later. Applicants respectfully submit that the fact that the Examiner has failed to identify a single reference that describes use of antifoam in a qRT-PCR during the 11 year period between 1991 and 2002, a time period when literally thousands of laboratories around the world were using qRT-PCR, is very telling. Either the art did not recognize that the presence of detergents adversely affected the accuracy of optical detection in RT-PCR, or did not recognize that antifoams surprisingly could be used in RT-PCR without inhibiting polymerase activity, or both. In any event, the Examiner has cited to no references that teach or suggest the use of antifoams in qRT-PCR and accordingly, no *prima facie* case of obviousness exists and the rejections should be withdrawn.

Lopez Garcia does not teach use of antifoams to improve optical detection

Lopez Garcia describes methods of determining the content of certain metals in ores by flame atomic absorption spectroscopy (flame AAS). Lopez Garcia does not teach or suggest qRT-PCR, let alone a qRT-PCR with improved optical detection. Lopez Garcia does not address the role of detergents in stabilizing thermostable DNA polymerases, nor does it teach or suggest that antifoams, which directly affect detergent action, can be used to prevent bubble formation and improve optical detection in qRT-PCR reactions without negatively impacting polymerase

[1] Holland *et al. Proc. Nat'l Acad. Sci.* USA 88:7276-7280 (1991); US Patent No. 5,994,056)

stability and activity. In fact, the logical relationship between the use of antifoam in Lopez Garcia and the use of the antifoam in the instantly claimed methods and compositions is so attenuated as to be non-existent.

Moreover, contrary to the Examiner's assertions, the use of antifoam in Lopez Garcia has *nothing* to do with the optical detection of the signal in the flame AAS. In fact, a careful reading of Lopez Garcia reveals that the attempts to use a combination of detergent and antifoam *failed* because the presence of detergent led to inaccurate and unreliable measurements. If by some remarkable chance one skilled in the art of qRT-PCR would somehow have come across Lopez Garcia, a publication in an entirely unrelated field, describing techniques used under conditions that are completely incompatible with qRT-PCR, that skilled artisan would recognize that the presence of detergents and antifoam can interfere with optical detection methods to the extent that they are no longer accurate.

Accordingly, Lopez Garcia could not provide one skilled in the art with a reasonable expectation of success in using combinations of detergent and antifoam in qRT-PCR, since it describes the failure of that combination of reagents to provide accurate AAS measurements, The Examiner has provided no rationale as to why one skilled in the art would deduce that a method that failed in AAS would reasonably be expected to succeed in a completely different technique under completely different conditions. In the absence of any such rationale, Lopez Garcia cannot teach or suggest use of antifoam to improve methods of optical detection in flame AAS, let alone in qRT-PCR.

In flame AAS a liquid sample is aspirated by a pneumatic nebulizer, transformed into an aerosol, and introduced into a spray chamber, where it is mixed with flame gases and aerosol droplets enter the flame. The atomic absorbance at specific wavelengths is then measured and the concentration of analyte determined using the venerable Beer-Lambert law. The temperature of the flame in flame AAS is in excess of 2000°C. It is clear that at this temperature, any aqueous liquid is in the gaseous state prior to any optical detection, and so bubbles cannot conceivably affect the optical detection - for the simple reason that it is physically impossible for aqueous bubbles to be present at 2000°C. If bubbles cannot affect optical detection in a flame AAS, it follows that antifoams cannot improve or reduce optical detection in flame AAS.

The Examiner seemingly recognizes this problem underlying the logical basis of the rejection and seeks to argue that "elimination of air bubbles before feeding the sample into the

nebulizer ensures continuous loading of sample into the nebulizer (and therefore, the optical path of the detection device), which results in improved accuracy." Applicants respectfully submit that there is no reasonable nexus between the use of antifoam and the optical detection in Lopez Garcia.

All that the flame AAS method described in Lopez Garcia requires is that a known amount of sample be introduced into the flame at a known rate *prior* to optical detection. Bubbles in the tubing containing the sample affect this rate, but do not affect the optical detection, because *there are no bubbles present* during optical detection (see above). The surprising effects of antifoam on increasing the accuracy of qRT-PCR detection that are observed in the instantly claimed methods and compositions are not caused by any effect on flow, and therefore the Examiner's citation of Lopez Garcia is wholly inapposite.

As an illustration that the effects of antifoam on accuracy of optical detection in qRT-PCR are not related to any effect on flow, Example 1 of the instant application describes qRT-PCR reactions that are carried out in a sealed container, where there is no flow, and therefore no effect of bubbles on the flow. The Examiner therefore has provided no rationale as to why the presence of antifoam would have been expected to lead to improved optical detection in qRT-PCR and no *prima facie* case of obviousness exists and the rejection should be withdrawn.

Stemmer does not teach or suggest use of antifoam in qRT-PCR

It is axiomatic that an Examiner must consider a reference as a whole, and may not ignore any part of a reference that would lead away from the claimed invention. MPEP §2141.02(VI), citing *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). In the instant case, the Examiner has failed to consider Stemmer as a whole, and has merely cherry-picked portions of Stemmer that support the rejection, while ignoring those parts of the reference that are in direct conflict with the interpretation that the Examiner places on Stemmer. When Stemmer is properly considered as a whole, it is clear that no *prima facie* case of obviousness exists and that the rejection is improper and should be withdrawn.

Stemmer is directed to so-called "DNA shuffling" methods that can be used to create and screen new proteins. These methods use PCR only to amplify the genes and gene segments produced using the shuffling methods. Nothing in Stemmer teaches or suggests uses of PCR that

are anything other than completely conventional, and one skilled in the art would not look to Stemmer to redefine in any way the conditions for PCR that were long established by the time Stemmer was filed.

Moreover, Stemmer specifically cites the conventional and well-known PCR conditions in a first paragraph at column 9, line 64, to column 10, line 6:

> As used herein, "suitable reaction conditions" are those conditions suitable for conducting PCR amplification using conventional reagents. Such conditions are known or readily established by those of skill in the art, and can be exemplified by the reaction conditions used in U.S. Pats. 4,683,202, 4,683,195, and 4,800,159, which are incorporated herein by reference. As one example and not to limit the invention, suitable reaction conditions can comprise: 0.2mM each dNTP, 2.2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCI pH 9.0, 0.1 % Triton X-100.

The patents cited in this paragraph are the famous Mullis patents that detailed the PCR process that led to Mullis being awarded the Nobel Prize. The conditions listed in this paragraph are the conditions used in millions of PCR reactions every year and the reagents match the contents of many commercial "mastermix" products for PCR. Moreover, these conditions are the conditions actually used in the working examples provided by Stemmer (see Example 1, columns 18 and 19), and Stemmer actually uses a commercial mastermix that substantially matches these conditions in the Examples[2].

The Examiner ignores this first paragraph in Stemmer, which cites conventional and workable PCR conditions, and instead relies on a second paragraph, immediately following the first paragraph, at column 10, lines 7-30. This second paragraph not only contradicts the first, immediately preceding paragraph, but is internally inconsistent and defines the use of conditions that one skilled in the art would be well aware are incompatible with PCR:

> As used herein the term "physiological conditions" refers to temperature, pH, ionic strength, viscosity, and like biochemical parameters which are compatible with a viable organism, and/or which typically exist intracellularly in a viable cultured yeast cell or mammalian cell. For example, the intracellular conditions in a yeast cell grown under typical laboratory culture conditions are physiological conditions. Suitable in vitro reaction conditions for PCR and many polynucleotide enzymatic reactions and manipulations are generally physiological conditions. In general, in vitro physiological conditions comprise 50-200 mM NaCl or KCl, pH

[2] The Li reference (cited by the Examiner to show that quantitative PCR was well established) also uses this type of commercial mastermix.

> 6.5-8.5, 20°-45° C. and 0.001-10 mM divalent cation (e.g., Mg++Ca++); preferably about 150 mM NaCl or KCl, pH 7.2-7.6, 5 mM divalent cation, and often include 0.01-1.0 percent nonspecific protein (e.g., BSA). A nonionic detergent (Tween, NP-40, Triton X-l00) can often be present, usually at about 0.001 to 2%, typically 0.05-0.2% (v/v). Particular aqueous conditions may be selected by the practitioner according to conventional methods. For general guidance, the following buffered aqueous conditions may be applicable: 10-250 mM NaCl, 5-50 mM Tris HCI, pH 5-8, with option of divalent cation(s) and/or metal chelators and/or nonionic detergents and/or membrane fractions and/ or antifoam agents and/or scintillants.

Unlike the first paragraph describing PCR conditions, Stemmer makes no further use of, or reference to, the putative conditions set forth in the second paragraph.

The Examiner ignores the obvious contradictions between this second paragraph and the preceding first paragraph, but relies on the reference to antifoam agents in the second paragraph and bases the entire rejection on the single sentence:

> Suitable in vitro reaction conditions for PCR and many polynucleotide enzymatic reactions and manipulations are generally physiological conditions.

Thus, the Examiner asserts that Stemmer teaches that PCR is compatible with antifoam reagents because PCR can be carried out under "physiological conditions" and Stemmer defines "physiological conditions" as including antifoam reagents.

This basis for the rejection runs afoul of MPEP §2141.02 (VI) because it improperly ignores all those parts of Stemmer that lead away from the use of antifoams in PCR. In particular, the Examiner ignores the fact that the conditions cited in the second paragraph are incompatible with PCR, as discussed in more detail below, and further ignores the fact that Stemmer provides no data to overcome the prejudice that antifoams would be expected to inhibit PCR by interfering with the action of detergent known to be needed for polymerase stability.

As a first point, one of ordinary skill in the art would recognize the contradiction between the broad definition of "physiological conditions" cited by Stemmer and the conditions of qRT-PCR. Thus, Stemmer defines "physiological conditions" as those conditions of temperature, pH, ionic strength, viscosity, and the like which are compatible with a viable organism, and/or which typically exist intracellularly in a viable cultured yeast cell or mammalian cell. On the other hand, PCR is well known as a process that involves repeatedly heating a sample to over 90°C and cooling it to approximately 60°C. These are not conditions that reasonably can be

understood to be "compatible with a viable organism" nor do they exist within a yeast or mammalian cell. If this rejection is to be maintained, applicants respectfully submit that the Examiner specifically explain why one skilled in the art would reasonably rely on a paragraph that begins with a statement of fact that is clearly and demonstrably incorrect.

Second, Stemmer's second paragraph then provides two sets of conditions that are "physiological conditions." Not only are these conditions different, but neither are compatible with PCR, and both differ from the conditions actually used in the working examples by Stemmer. The table below lists and compares each of the conditions provided by Stemmer, and shows the incompatibilities between the conditions recited in the second paragraph and those actually used by Stemmer:

Component	**First set of conditions**	**Second set of conditions**	**PCR conditions in Example 1**
Salt concentration	50-200 mM NaCl or KCl, preferably 150mM	10-250 NaCl	50 mM KCI
pH	pH 6.5-8.5, preferably 7.2-7.6	pH 5-8	pH 9.0
Temperature	20°-45° C	unspecified	94° C. for 30 seconds, 50°-55° C. for 30 seconds, 72° C. for 30 seconds and 5 minutes at 72° C
Divalent cation	0.001-10 mM divalent cation (e.g., $Mg^{2+}Ca^{2+}$), preferably 5 mM	Optional, unknown identity or quantity	2.2mM $MgCl_2$
Buffer	None specified	5-50 mM Tris	10 mM Tris
Added non-specific protein	often include 0.01-1.0 percent nonspecific protein (e.g., BSA).	Not mentioned	absent
Detergent	Nonionic detergent (Tween, NP-40, Triton X-100) optional, usually at about 0.001 to 2%, typically 0.05-0.2% (v/v).	Optional, unknown identity or quantity	0.1% Triton X-100
Chelators	Unspecified	Optional, unknown identity or quantity	absent

Antifoam	Unspecified	Optional, unknown identity or quantity	absent
Scintillant	Unspecified	Optional, unknown identity or quantity	absent

The second paragraph therefore suggests use of two different pH ranges, neither of which is close to the pH actually used by Stemmer. It suggests temperature conditions that clearly would not permit a PCR to occur. It either fails to specify the presence of Mg ion, or specifies broad concentration ranges with a preferred concentration that is well known to be incompatible with PCR (since PCR is known to be highly sensitive to Mg ion concentration). It suggests that chelators, which bind Mg ion, are compatible with PCR when it is known that PCR is highly sensitive to Mg ion concentration. It suggests that scintillants may be used in PCR reactions, whereas the chemical nature of scintillants would be expected to be incompatible with PCR. Given these problems with the second paragraph, why would one of ordinary skill in the art reasonably have relied on it for the source of PCR reagents and/or conditions? The Examiner fails to explain the clear incompatibilities between the conditions defined in the second paragraph and the conventional PCR conditions actually used by Stemmer.

One skilled in the art reading the second paragraph would either: (a) try the proposed conditions and find that these conditions do not work for PCR (with or without antifoam) or, more likely (b) recognize that the cited conditions are incompatible with PCR and dismiss the second paragraph as not being a reliable source for experimental conditions. In either case, this leads to the inexorable conclusion that the second paragraph *could not* provide a reasonable expectation of success in using an antifoam in PCR, because the conditions provided in which an antifoam is listed *do not work* for PCR.

Moreover, one of ordinary skill in the art would be aware of the role that detergents play in PCR by stabilizing the polymerase and preserving its activity, and also would have been aware that antifoams would be expected to interfere with the detergent action and therefore would have been expected to negatively affect polymerase activity. The Examiner cannot overcome this prejudice against the use of antifoams merely by relying on a paragraph in Stemmer that mentions antifoam in the context of conditions that are clearly incompatible with PCR without explaining in detail why one of ordinary skill in the art would reasonably rely on one passing reference to antifoam among a welter of references to reagents that are incompatible with PCR.

The Examiner's position here clearly is incompatible with the requirements of MPEP 2041.02(VI) that the reference be viewed as a whole. The Federal Circuit has long recognized the improper nature of "cherry picking" in a reference only so much as to support an obviousness rejection:

> [i]t is impermissible within the framework of section 103 to pick and choose from any one reference only so much of it as will support a given position to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one skilled in the art. (*Bausch & Lomb, Inc., v. Barnes-Hind/Hydrocurve, Inc.*, 796, F.2d 443, 448 (Fed. Cir. 1986) (quoting *In re Wesslau*, 355 F. 2d 238, 241 (CCPA 1965)).

Nothing in Stemmer teaches or describes quantitative PCR, as recited in the instant claims, and nothing in Stemmer would have provided one of ordinary skill in the art with a reasonable expectation of success in using an antifoam reagent in qRT-PCR. Accordingly, no *prima facie* case of obviousness exists and the rejection is improper and should be withdrawn.

The secondary references fail to cure the deficiencies of Li, Stemmer and Lopez Garcia

None of the secondary references do anything to cure the multiple deficiencies of the primary references as set forth above. Blaschke is cited only to show that real-time RT-PCR can use either TaqMan probes or nucleic acid-binding dyes, and is silent both as to the potential for interference with optical detection and the use of antifoams to prevent this interference. Kyle is cited merely to show the use of antifoam 1520-US in a conventional fermentation (non-PCR) application. Wierenga merely shows that combinations of antifoams can provide higher antifoam properties in laundry and dishwasher applications than single antifoams, and teaches nothing about PCR reactions, let alone the use of antifoams in PCR applications. The Sigma catalogue is cited only to show that combinations of organic and silicone-based anti-foams are known and, like Blaschke, Kyle and/or Wierenga, fails to teach or suggest the use of antifoams in real-time quantitative PCR reactions. None of the secondary references, alone or in any combination with the primary references, teach or suggest the methods or compositions of the instant claims. For at least these reasons, Applicants respectfully submit that no *prima facie* case of obviousness has been presented.

The Examiner also has cited to two patents (US 5962273 and US 5985569) that refer to the use of an unspecified antifoam in the context of an isothermal strand displacement reaction.

Applicants respectfully point out that the isothermal reaction described in those patents uses Bst polymerase that is not thermally stable under PCR conditions and is not compatible with PCR[3]. Moreover the strand displacement reaction does not use optical detection. Finally, these patents are cited in the specification of the instant application (see paragraphs 15 and 35 of the published application US 2005/0123924 Al) and the use of Bst polymerase is specifically excluded from the scope of the instant claims. Nothing in either patent provides any information that would overcome the prejudice in the art against the use of antifoams in PCR as described in detail above.

[3] The *New England Biolabs* catalogue states, for example, that Bst polymerase is"not recommended at temperatures greater than 70°C because it becomes heat-inactivated."

Conclusion

In view of the foregoing remarks, Applicants respectfully submit that the application is in condition for allowance. Should the Examiner feel that there are any issues outstanding after consideration of this response, the Examiner is invited to contact the undersigned to expedite prosecution of the application.

The Commissioner is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-2283. **This paragraph is intended to be a CONSTRUCTIVE PETITION FOR EXTENSION OF TIME in accordance with 37 C.F.R. § 1.136(a)(3).**

Respectfully submitted,

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